CLAIMS

What is claimed is:

1. A method for generating a secreted trimeric fusion protein, comprising:

(a) creating a DNA construct comprising a transcriptional promoter linked to a template encoding a signal peptide sequence followed by in-frame fusion to polypeptide to be trimerized, which in turn is joined in-frame to a polypeptide capable of self-trimerization which is heterologous from the first polypeptide to be trimerized; (b) introducing said DNA construct into a eukaryotic cell; (c) growing said host cell in an appropriate growth medium under physiological conditions to allow the secretion of a trimerized fusion-protein encoded by said DNA sequence; (d) isolating said trimerized fusion protein from the culture medium of said host cell.

2. The method of claim 1 wherein the trimerized polypeptide fusion is a homotrimer.

3. The method of claim 1 wherein the trmerizing polypeptide moiety comprises the C terminal portion of collagen capable of self-assembly into a trimer selected from the group consisting of pro.alpha.1(I), pro.alpha 2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI).

4. A method for generating a secreted trimeric fusion protein, comprising:

(a) introducing into a eukaryotic host cell a first DNA construct comprising a promoter which drives the transcription of an open reading frame consisting of a signal peptide sequence which is linked in-frame to a non-collagen polypeptide to be trimerized, which

in turn is joined in-frame to the C-terminal portion of collagen capable of self-trimerization, selected from pro.alpha.1(I), pro.alpha.2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI); (b) introducing into a eukaryotic host cell a second DNA construct comprising a promoter which drives the transcription of an open reading frame consisting of a second signal peptide sequence which is linked in-frame to a second non-collagen polypeptide to be trimerized, which in turn is joined in-frame to the second C-terminal portion of collagen capable of self-trimerization, selected from pro.alpha.1(I), pro.alpha.2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI); (c) growing the host cell in an appropriate growth medium under physiological conditions to allow the secretion of a trimerized fusion protein encoded by said first and second DNA sequences; and (d) isolating the secreted trimeric fusion protein from the host cell.

5. A method for generating a secreted trimeric fusion protein, comprising:

(a) introducing into a eukaryotic host cell a first DNA construct comprising a promoter which drives the transcription of an open reading frame consisting of a signal peptide sequence which is linked in-frame to a non-collagen polypeptide to be trimerized, which in turn is joined in-frame to the C-terminal portion of collagen capable of self-trimerization, selected from pro.alpha.1(I), pro.alpha.2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI); (b) introducing into a eukaryotic host cell a second DNA construct comprising a promoter which drives the transcription of an open reading frame consisting

of a second signal peptide sequence which is linked in-frame to a second non-collagen polypeptide to be trimerized, which in turn is joined in-frame to a second C-terminal portion of collagen capable of self-trimerization, selected from pro.alpha.1(I), pro.alpha.2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI); (c) introducing into a eukaryotic host cell a third DNA construct comprising a promoter which drives the transcription of an open reading frame consisting of a third signal peptide sequence which is linked in-frame to a third non-collagen polypeptide to be trimerized, which in turn is joined in-frame to a third C-terminal portion of collagen capable of self-trimerization, selected from pro.alpha.1(I), pro.alpha.2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI); (d) growing the host cell in an appropriate growth medium under physiological conditions to allow the secretion of a trimerized fusion protein encoded by said first and second DNA sequences; and (e) isolating the secreted trimeric fusion protein from the host cell.

6. The methods of claims 1-5, wherein the signal peptide sequence and the non-collagen polypeptide to be trimerized are both from the same native secreted protein.

7. The methods of claims 1- 5, wherein the signal peptide sequence and the non-collagenous protein to be trimerized are selected from two different secreted proteins.

8. The methods of claim1, 4 and 5, wherein the host eukaryotic cell is a fungal or insect cell.

9. The methods of claim 1, 4 and 5, wherein the host eukaryotic cell is a cultured mammalian cell line.

10. The methods of claims 1-5, wherein a C-terminal portion of collagen includes a "glycine-repeat" triple helical region of collagen linked to a C-propeptide.

11. The methods of claim 10, wherein a C-terminal portion of collagen is identified by Sequence ID Nos. 1-2.

12. The methods of claims 1-5, wherein the trimerizing C-terminal portion of collagen comprises only a C-propeptide without any glycine-repeat triple helical region of collagen.

13. The methods of claims 10 -12, wherein the trimerizing C-terminal portion of collagen comprises a mutated or deleted BMP-1 protease recognition sequence, thereby conferring the fusion proteins resistance to said protease degradation.

14. The methods of claims 12-13, wherein the trimerizing C-terminal portion of collagen is identified by sequence ID Nos. 3-4.

15. Compositions of fusion proteins generated by the methods of claims 1, 2, 3, 10, 11, 12, 13 and 14 are soluble trimeric human TNF-α receptor II (p75) identified by Sequence ID Nos. 9-12.

16. Compositions of fusion proteins generated by the methods of claims 1, 2, 3, 10, 11, 12, 13 and 14 are soluble trimeric human CD4 identified by Sequence ID Nos. 13-16.

17. Compositions of fusion proteins generated by the methods of claims 1, 2, 3, 10, 11, 12, 13 and 14 are soluble trimeric soluble human placental alkaline phosphatase identified by Sequence ID No. 5-8.

18. A trimerized polypeptide fusion comprising three polypeptide chains, each of said chains comprises a ligand-binding domain of a receptor joined to a C-propeptide of collagen, wherein trimerization of the polypeptide fusion results in enhancement of biological activity.

19. A method of blocking TNF-α biological activity using a trimerized soluble TNF-α receptor II generated by claim 15.